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2020-10

Clough , T J , Rochette , P , Thomas , S M , Pihlatie , M , Christiansen , J R & Thorman , R E 2020 , ' Global Research Alliance N2O chamber methodology guidelines: Design considerations ' , Journal of Environmental Quality , vol. 49 , no. 5 , pp. 1081-1091 . https://doi.org/10.1002/jeq2.2011

http://hdl.handle.net/10138/327500
https://doi.org/10.1002/jeq2.20117

Received: 14 October 2019 | Accepted: 11 April 2020 | Published online: 23 July 2020

DOI: 10.1002/jeq2.20117

Journal of Environmental Quality

SPECIAL SECTION:
GRA N_2O CHAMBER METHODOLOGY GUIDELINES

Global Research Alliance N_2O chamber methodology guidelines: Design considerations

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Assigned to Associate Editor Cecile de Klein.

Abstract

Terrestrial ecosystems, both natural ecosystems and agroecosystems, generate greenhouse gases (GHGs). The chamber method is the most common method to quantify GHG fluxes from soil–plant systems and to better understand factors affecting their generation and mitigation. The objective of this study was to review and synthesize literature on chamber designs (non-flow-through, non-steady-state chamber) and associated factors that affect GHG nitrous oxide (N_2O) flux measurement when using chamber methods. Chamber design requires consideration of many facets that include materials, insulation, sealing, venting, depth of placement, and the need to maintain plant growth and activity. Final designs should be tailored, and bench tested, in order to meet the nuances of the experimental objectives and the ecosystem under study while reducing potential artifacts. Good insulation, to prevent temperature fluctuations and pressure changes, and a high-quality seal between base and chamber are essential. Elimination of pressure differentials between headspace and atmosphere through venting should be performed, and designs now exist to eliminate Venturi effects of earlier tube-type vent designs. The use of fans within the chamber headspace increases measurement precision but may alter the flux. To establish best practice recommendations when using fans, further data are required, particularly in systems containing tall plants, to systematically evaluate the effects that fan speed, position, and mixing rate have on soil gas flux.

Abbreviations: GHG, greenhouse gas; PVC, polyvinyl chloride.

1 | INTRODUCTION

Chamber designs may use flow-through, non-steady-state, or steady-state chambers (Denmead, 1979), or non-flow-through, non-steady-state chambers (Rochette & Eriksen-Hamel, 2008). However, the literature on nitrous oxide (N_2O) emissions is dominated by the use of non-flow-through, non-steady-state chamber methodologies (Bouwman, Boumans, & Batjes, 2002), often referred to as "static chambers."

Since chambers are invasive, nuances in chamber design can affect the accuracy of N_2O flux determination (Parkin, Venterea, & Hargreaves, 2012; Pavelka et al., 2018), and the subsequent upscaling of results. This is because chambers can change the vertical diffusion of N_2O in the soil, the soil energy balance, and degree of turbulence above the soil (Rochette, 2011). This article provides recommendations on the minimum requirements, and discusses the key principles, for chamber designs to minimize the impact of the measurement technique on the natural soil and atmospheric processes. It provides guidance and recommendations on materials, dimensions, venting, seals, insulation, sampling port, plant effects, and headspace mixing.

Core Ideas

- Chamber construction and design are critical for accurate soil gas flux determination.
- Ecosystem, soil conditions, and experimental aims dictate design.
- Understanding possible plant effects on N_2O fluxes requires sophisticated design.
- Data on the potential artifacts of headspace mixing using fans are still needed.

2 | CHAMBER MATERIALS AND METHODS

Above all, chamber materials should not react with or consume the gas of interest within the plant–soil system. Neither should they emit any contaminants into the atmosphere above the soil surface, nor the soil itself, once positioned. Recommended materials for chambers used to determine greenhouse gas (GHG) fluxes from soils so far include stainless steel, aluminum, polyvinyl chloride (PVC), polycarbonate, polyethylene, or polymethyl methacrylate (Plexiglas, acrylic sheet) (Parkin & Venterea, 2010). Other factors, such as the presence or absence of plants, may also influence the choice of material, as discussed below.

Any other components used in chamber construction, such as seals, tubing, septa, and vents, should also be inert. The chamber system should also be robust. If used in grazed pasture studies, chamber materials must be rigid so as to prevent chamber flex, and to withstand treading and chewing by grazing animals. Heavy mesh cages may be needed to stop cattle damaging chambers.

In the past, chambers have been as simple as "push-in covers" pressed into the soil. The insertion of the push-in chambers has been demonstrated to disturb the soil structure along with roots and biota in the soil profile. Hence, this affects gas transport and production processes and induces pressure changes (piston flow of air) as a result of chamber placement (Hutchinson & Livingston, 2001; Matthias, Blackmer, & Bremner, 1980). Such chambers should not be used. Instead, chambers should consist of a paired "base–chamber" design, where the chamber is placed and sealed onto a base—sometimes also referred to as an "anchor" or "soil collar" (Parkin & Venterea, 2010)—previously inserted into the soil. Depending on the application, insertion time before the flux measurements begin can vary from hours for a bare-textured soil to weeks when insertion results in root damage. In forest ecosystems, root-damage-induced artifacts must be avoided and soil collars may be sealed against the soil surface. Other considerations around chamber design and dimensions, as discussed below, are venting, sample ports, effective sealing, soil temperature monitoring, and insulation.

3 | CHAMBER DIMENSIONS

Chamber design can be considered as being ecosystem specific, requiring customized solutions. Good chamber design must consider certain critical dimensions, such as the internal chamber height above the soil surface, the chamber area (cm^2), and the length (cm) of the chamber perimeter. These last two factors are used to calculate the chamber area/perimeter ratio, which Rochette and Eriksen-Hamel (2008) recommended should be $\geq$10 cm (e.g., a cylindrical chamber of 40-cm diam.), based on work by Healy, Striegel, Russell, Hutchinson, and Livingston (1996). This is because the relative error associated with any poor chamber seal decreases as the diameter of a chamber increases. Similarly, Pihlatie et al. (2013) also found that small chambers with an area/perimeter ratio <9 cm (height $\leq$0.22 m, area $\leq$0.10 m^2, and volume $\leq$0.015 m^3) were prone to underestimate gas fluxes regardless of flux calculation method; however, with an area/perimeter ratio >9 cm (height >0.22 m, area >0.10 m^2, and volume >0.015 m^3), the fluxes were underestimated only with the linear flux calculation method. Hoffmann et al. (2018) also demonstrated an effect of varying chamber size and geometry, using carbon dioxide (CO_2), finding that small light chambers with a rubber seal were more prone to leakage. Chamber area will depend on the ecosystem in which the apparatus is deployed: larger chambers can of course be placed on relatively flat, clear terrain, but forest ecosystems might require chambers of smaller area. In either case, the chamber will ideally be as large as feasibly possible in order to capture spatial variation. Chambers covering an area up to 2 m^2 have been used, but most common models have an area smaller than 0.5 m^2.

A chamber's geometry is important when dealing with spatial variability problems at small scales (Rochette & Hutchinson, 2005). For example, in a row crop, nitrogen fertilizer banding and soil compaction in the inter-rows often produce a flux gradient perpendicular to the plant rows. If a research objective is to describe that gradient, long, narrow, rectangular chambers are most appropriate. Thus, consideration of chamber geometry is required and circular chambers should be avoided. If a description of the inter-row gradient flux is unnecessary, then chambers covering the whole inter-row are most efficient. Olfs et al. (2018) noted that placement of chambers in inter-row zones may lead to bias due to the omission of the rhizosphere or banded fertilizer zones. Thus, they produced a new split-chamber design for measuring N_2O fluxes within the row of a maize (*Zea mays* L.) crop, where stalks of the growing maize plants are clamped between rubber strips to create a seal. This design was carefully bench tested for airtightness prior to successful field testing (Olfs et al., 2018). In grazed pasture systems, chambers are often circular, so as to enclose the generally circular area of animal urine patches. Smaller chambers may also be required, particularly for studies exploring the spatial variability of fluxes (e.g., the effect of animal hoof compaction vs. noncompacted soil surrounding the hoof print).

Height is another critical feature of chamber design. As chamber height increases, the impact on environmental variables such as humidity, or the N_2O diffusion gradient within the soil, is reduced. However, the minimum detectable flux increases (Hutchinson & Livingston, 2002; Rochette & Eriksen-Hamel, 2008). Conversely, if the chamber height is decreased, the minimum detectable flux is lower, but at the expense of greater perturbation of the system (temperature, humidity, and gas concentration). The significance of these perturbations—and their dependence on chamber height—is intrinsically linked to chamber deployment time, so Rochette and Eriksen-Hamel (2008) devised a ratio of chamber height (cm) to deployment time (h), recommending that well-designed and deployed chambers have a ratio of $\geq$40 cm h^{-1}.

The ecosystem environment, expected flux rates, and size of the plants strongly affect the choice of chamber design, size, and material. If the aim is to capture the role of a tall plant, such as wheat (*Triticum aestivum* L.), this will dictate the chamber height. However, the user needs to be aware that detectable fluxes will be lower, so the closure period may need to be extended. Also, uniform N_2O concentrations may not be present within the chamber at time of sampling (see below). One option is to use chamber extensions. These are sections used to extend the height of the chamber as the plant grows, but care needs to be taken with the seal between chamber extensions, and mixing of the headspace, particularly around extensive crop canopies. Leaks can be difficult to detect. However, if CO_2 is measured simultaneously with N_2O, it may provide supportive evidence of leakage: if the headspace CO_2 concentration does not increase in the absence of plants, or fails to decline in the presence of plants, then it can be assumed a leak is present.

Atypical soil moisture conditions within the chamber must be avoided, because water retention in the base after rain or irrigation could change soil aeration, soil temperature, and microbial processes. Although the chamber base is left open between the flux measurements, the base must be as shallow as possible to avoid shading, since this could change soil temperature and lead to unintended effects on soil moisture and microbial processes. Parkin and Venterea (2010) and Pavelka et al. (2018) recommend base walls no higher than 5 cm: however, chamber bases can be designed to be at the soil surface (Parkin & Venterea, 2010).

Another critical dimension is the collar insertion depth into the soil. Failure to push it deeply enough into the soil can allow N_2O to leak, or ambient air to contaminate the chamber headspace via lateral diffusion of gases through the soil, as a consequence of the vertical N_2O concentration gradient being disrupted (Rochette & Eriksen-Hamel, 2008). To prevent artifacts, and when possible, the base walls need to be inserted to at least the depth where N_2O concentrations are not being perturbed by feedback effects of the chamber, so as to prevent lateral diffusion of N_2O beneath the wall. (Healy et al., 1996; Hutchinson & Livingston, 2001). In natural environments such as forest soils, the cutting of the roots of trees and ground vegetation introduces an additional error to the flux measurements. Decaying roots may influence the carbon and nitrogen turnover in the soil, and this effect should be accounted for, especially if the aim is to follow interactions between tree roots and soil microbial processes. In those cases, the airtightness of the chamber base should be ensured by additional sealing material on the outer side of the chamber base (e.g., by inert fine sand or clay).

Hutchinson and Livingston (2001) modeled the relationship between deployment time, air-filled porosity (0.1, 0.3, and 0.5 cm^3 cm^{-3}), and the base insertion depths required to reduce lateral diffusion by either 1 or 5% of the steady-state N_2O flux. Their results indicated that a 5-cm insertion depth was more than sufficient in soil with low effective diffusivity (soil air-filled porosity ≤0.1 cm^3 cm^{-3}). However, it was only adequate for brief deployment periods (20–30 min) at a soil porosity of 0.3 cm^3 cm^{-3}, and inadequate at higher values of soil air-filled porosity (0.5 cm^3 cm^{-3}). Their data indicate that for deployment times of 30 min, insertion depth should be 10 cm at a soil air-filled porosity ≤0.3 cm^3 cm^{-3}, increasing to 20 cm if air-filled porosity is as high as 0.5 cm^3 cm^{-3}.

Rochette and Eriksen-Hamel (2008) concluded in their review study that a ratio of insertion depth to deployment time of ≥12 cm h^{-1} was very good. A prior knowledge of maximum soil air-filled porosities at the site of chamber deployment can help reduce errors, and the data of Hutchinson and Livingston (2001) should be consulted for guidance.

4 | VENTING

Pressure changes within chamber headspace during chamber placement, gas sampling, or the measurement due to external wind or changes in temperature can critically affect the gas fluxes. Placing a vent in the chamber to control and avoid the pressure changes is hence recommended. Published evidence clearly supports and recommends the venting of non-steady-state chambers (Bain et al., 2005; Davidson, Savage, Verchot, & Navarro, 2002; Hutchinson & Livingston, 2001; Hutchinson & Mosier, 1981; Xu et al., 2006). Vents prevent pressure gradients between the interior and exterior of the chamber from influencing gas exchange. Pressure gradients can occur when the chamber is placed on its base, during the sampling of the chamber headspace (Christiansen, Korhonen, Juszczak, Giebels, & Pihlatie, 2011), or if the chamber heats or cools suddenly. Pressure-induced errors during the placement of a chamber can be avoided if a sample port or a sufficiently sized vent-tube is kept open during the chamber placement (Christiansen et al., 2011). Inadequate insulation may cause pressure differentials to develop in unvented chambers, as a result of cooling or warming of the chamber air (Davidson et al., 2002). Naturally occurring pressure gradients may occur outside the chamber as a result of wind-driven turbulence (Rochette, 2011). If the turbulence-driven changes in barometric pressure are reduced due to a chamber's placement over the soil surface, N_2O emissions will be reduced inside the chamber (Hutchinson & Mosier, 1981).

Higher N_2O fluxes have been reported when vents have been used in chambers (Hutchinson & Mosier, 1981). In another study, the use of vents increased measured N_2O fluxes fivefold in a well-aerated soil but reduced them in more impermeable soils, suggesting that vents might create greater problems than they solve (Conen & Smith, 1998). However, well-designed vents transmit barometric pressure fluctuations while minimizing leaks (i.e., N_2O diffusion out of the chamber via the vent tube) and contamination (i.e., the intake of external ambient air into the chamber during gas sampling, or temperature-induced pressure changes inside the chamber).

Vents have previously been constructed from inert tubing and secured through the chamber wall with an appropriate gastight bulkhead fitting. Criteria for optimal vent design, given by Hutchinson and Mosier (1981), stated that (a) the tube diameter (D) must be small enough to minimize diffusive losses, but large enough to permit air—moving in response to pressure changes—to flow down the tube with pressure loss no greater than 0.1 μbar, and (b) the vent tube length (L) must be not less than that which gives an internal volume five times greater than the volume of enclosed air displaced by the largest anticipated pressure wave.

The equations provided by Hutchinson and Mosier (1981), which relate wind speed, D, and L, must be used to calculate the optimum vent tube dimensions for the conditions of the chamber study, such that the loss of accumulating N_2O by diffusion never exceeds 1% (Hutchinson & Mosier, 1981). A further practical guide to selecting vent tube length and diameter as a function of chamber volume and wind speed, based on Hutchinson and Mosier (1981), is provided by Parkin and Venterea (2010).

However, the use of vent tubes has also been shown to potentially induce a further source of error, due to wind flowing over the vent outlet and creating a Venturi effect that depressurizes the chamber (Bain et al., 2005; Conen & Smith, 1998; Suleau, Debacq, Dehaes, & Aubinet, 2009; Xu et al., 2006). Hutchinson and Livingston (2001) noted that the explanation for the results obtained by Conen and Smith (1998) was inconsistent. Because large pressure differences also occurred between vented and nonvented chamber types when wind speed and soil air permeability were smallest, they wondered if temperature-driven expansion had caused an effect. Davidson et al. (2002) noted that there were possible artifacts in both directions for the vented and nonvented chambers used by Conen and Smith (1998), making it difficult to know which chamber yielded the "true" flux. Davidson et al. (2002) did, however, measure an average internal chamber headspace pressure difference of −0.2 kPa on a moderately windy day, when a vented chamber was over a soil surface, but summarized their findings by stating that errors due to chamber pressure artifacts can be minimized—or almost eliminated—by appropriately sized vents. Hutchinson and Livingston (2001) stated that the weight of evidence is in favor of vents, and that, so long as vents are adequately designed, adverse effects are minimized. Potential Venturi effects can be further minimized by correctly sizing the vent—by mounting it as close as possible to ground level to minimize wind speed and by pointing the vent outlet downwind, and maybe even shielding it in strong winds. Bain et al. (2005) confirmed the Venturi effect described by Conen and Smith (1998) using flow-through, non-steady-state chambers (5 L PVC with vertical vent tube [0.19-cm diam., 3.56 cm long]) attached to either impermeable bases, or a PVC collar inserted 2–4 cm into the soil. For the chambers on the impermeable bases, the fan-controlled wind conditions in the field resulted in negative chamber pressures with a ~1 Pa drop in pressure per 1-m s^{-1} increase in horizontal wind speed at chamber height. When this was repeated on natural soil, no pressure changes occurred inside the chambers. All experimental variables were similar, and a negative pressure should have been induced. Bain et al. (2005) concluded that mass flow of gas through the soil was occurring and compensating for the chamber pressure gradient. Hoffmann et al. (2018) found that, in the case of a non-airtight chamber–collar interface, gas leakage was exacerbated by use of the vent and a fan.

Advection of a soil gas with this flow will increase the estimated gas flux. This same effect was observed by Xu et al. (2006), who recorded no negative pressures inside a chamber placed on a collar sitting on soil but found higher CO_2 fluxes in windy conditions. They subsequently found negative chamber pressures when the chamber was connected to an impermeable base. The lack of negative pressures with the chamber placed on the soil was due to mass flow of soil air into the chamber headspace. Such wind effects on mass flow will vary with soil moisture and porosity, and associated error will also depend on gas concentration (Xu et al., 2006). Suleau et al. (2009) found that locating vents (of their own design) 0.05 m above the soil surface was effective in reducing previous overestimates of flux (≤300%), which occurred in strong winds.

The Venturi effect has been overcome by improved vent design that virtually eliminates the occurrence of artificially induced pressures changes (−15 to 8 kPa) under windy conditions of up to 6.5 m s^{-1} (Xu et al., 2006). With wind speeds up to 4 m s^{-1} at chamber height Xu et al. (2006) showed that flux overestimates of up to 19% occurred in CO_2 flux calculations when the soil CO_2 flux ranged from 0.5 to 2.5 μmol^{-2} s^{-1}.

In an investigation into N_2O emissions from manure-affected soil, Parker et al. (2017) tested three vents that included a commercially available vent (Licor Biosciences, consisting of two aluminum concentric plates, with a tapered cross section as described by Xu et al., 2006) and two others also fabricated with tapered profiles. These were evaluated at various plate spacings (h_1 = distance between plates at the perimeter, h_2 = inside distance between plates at the center) and compared with an open pipe vent. With peak wind speeds of 2.5–4.0 m s^{-1}, the open vent, situated in a vertical position on top of the chamber, repeatedly resulted in a Venturi effect with consistently negative pressure inside the chamber (−8 to −10 Pa). However, the Li-Cor vent with an h_1/h_2 ratio of 1/5 as recommended by Xu et al. (2006) reduced negative pressures from 0 to −0.5 Pa.

To ascertain whether a particular chamber-vent design does or does not invoke pressure gradients (Venturi effect), at wind speeds expected under field conditions, the chamber must be bench tested (e.g., Hoffmann et al., 2018) by sealing the base to an impermeable surface while wind speeds and internal chamber pressures are monitored (Bain et al., 2005; Hoffmann et al., 2018; Suleau et al., 2009; Xu et al., 2006).

5 | SEALS

An essential element of a multiple component chamber is the gastight seal placed between the two components. Hoffmann et al. (2018) suggested that the integrity of the sealing mechanism can be more important than specific technical issues such as the use of fans to mix the headspace and vents. A gastight seal is commonly achieved by placing a rubber gasket between the chamber and its base (Parkin & Venterea, 2010) or using a built-in trough, attached to the base, that holds water and acts as a seal between the two components (Christiansen et al., 2011; Hutchinson & Livingston, 2001). Specifications for the material(s) required to form the perfect seal between components have never been clearly defined. Obviously, the aim is to prevent N_2O leaking out of the chamber and external air into the chamber. Modeling by Hutchinson and Livingstone (2001) clearly showed that gasket material must have a very low internal cross-sectional area available for diffusion (i.e., a very low diffusivity) and must be pliable enough to form a good seal when compressed. Hutchinson and Livingston's (2001) simulation used a 0.25-cm-wide by 0.25-cm-high foam gasket that, at simulated porosities of 0.001–0.03, provided gas losses equal to 0.055 and 2.3% of the total mass flux, respectively.

In all cases, the modeled gas loss was greater through the simulated leaking gasket than through the vent (sized for a wind speed of 4 m s^{-1}; Hutchinson & Mosier, 1981), which was only 0.038% of the total mass flux. The study also highlighted the importance of eliminating gaps between the abutting seal and the component, with gaps as small as 1.2–53 μm resulting in the same loss of gas flux as achieved through diffusion through the gasket at simulated porosities of 0.001–0.03, respectively. This stresses the importance of having chamber components machined to high degrees of tolerance. Some form of fastener is often used to compress the chamber against the base's gasket, ensuring a tight seal. A seal's effectiveness can be tested by placing concentrations of a reference gas inside a chamber sealed to an impermeable surface and measuring the rate of N_2O concentration change over time. Ideally, there should be no changes in gas concentrations over typical deployment periods. Water seals have their shortcomings: they are only useful on flat ground, and they can dry out and can become dirty with algal growth. A supply of clean water is required at each sampling, and care must be taken not to spill water into the chamber, where it could affect the potential for N_2O production. Otherwise, water seals are very effective and a generally preferred option for flat sites.

6 | INSULATION AND TEMPERATURE CONTROL

Soil temperature affects the rates of N_2O production and consumption, the solubility of N_2O in soil water, and the diffusion rate of N_2O. Likewise, if the chamber is not vented, any temperature decreases or increases in the chamber will lead to negative or positive pressure effects, respectively (Rochette & Hutchinson, 2005). Parkin and Venterea (2010) calculated that, if not corrected for, significant temperature changes ($>5\,^\circ C\ h^{-1}$) will produce errors in calculated fluxes. Xu et al. (2006) noted that according to the ideal gas law, a 1 °C change in chamber temperature could result in a 333-Pa change in chamber pressure, with such an effect potentially causing fluxes to be underestimated. Chamber placement can alter soil temperature and thus biological processes that produce or consume N_2O.

Any increase in the concentration of other gases, resulting from headspace temperature changes, can affect N_2O concentrations (Rochette & Hutchinson, 2005). For example, Parkin and Venterea (2010) demonstrated how an increase in water vapor concentration—a consequence of increasing temperature elevating humidity in the headspace—could decrease N_2O concentrations by 3% (this is known as the water vapor dilution effect). This in itself may not cause an underestimation of the N_2O flux, since the final effect will depend on other factors, such as linearity of the flux over time. An increase in soil temperature may also enhance soil respiration, resulting in an increased demand for oxygen that may indirectly affect N_2O production mechanisms.

The aim of insulating the chamber, then, is to preserve and maintain the initial air and soil temperature present at the time of chamber placement. This may be achieved by coloring transparent chamber walls with Wite-Out (Pavelka et al., 2018) or covering the chamber, outer walls, and dorsal surface with either a reflective foil or an insulating material, or preferably a combination of both. Regardless of method, it must be proven satisfactory by comparing measured air and soil temperatures inside and outside the chamber during typical deployment periods and conditions.

Where plants are present, chamber studies may use transparent covers: these create significant problems with maintaining internal chamber temperatures and the design becomes more sophisticated due to the need for controlling temperature and its associated effects. Temperature control mechanisms can be implemented, for example, by installing heat exchangers, but they are expensive and can lead to other issues such as condensation. In their absence, flux measurement periods need to be kept short to minimize temperature effects, and temperatures need to be monitored.

7 | SAMPLING PORT

A sampling port is required to remove a gas sample from the chamber. It should be inert and gastight, except when samples are taken. Butyl rubber septa and syringe taps sealed to the chamber are often used. Septa materials must be inert and replaced at regular intervals to prevent leaks. The use of syringe taps may create "dead" air spaces that remain unexposed to the increasing gas concentration in the headspace. Care must be taken to purge these during the gas sampling process. Sampling ports can also be connected to a tube that samples air at several locations within the headspace to minimize problems associated with concentration gradients; again, these must be purged during the gas sampling process. Parkin and Venterea (2010) used a simple manifold built into the chamber cover to draw headspace air from four quadrants during sampling, in order to minimize any effect of gas concentration gradients in the headspace. However, few studies have used such systems.

8 | ALLOWING FOR PLANT EFFECTS

Plants can have significant effects on N_2O fluxes (Chang, Janzen, Cho, & Nakonechny, 1998; Jørgensen, Struwe, & Elberling, 2012; Pihlatie, Ambus, Rinne, Pilegaard, & Vesala, 2005; Reddy, Parick, & Lindau, 1989; Yu and Chen, 2009), and the chamber design may influence the rate of such effects on the N_2O flux. For example, placing an opaque chamber cover on the soil surface, over the top of plants, will block incoming radiation, which in turn influences plant physiological activity (e.g., leading to stomatal closure; Hopkins & Hüner, 2009). This can reduce the transpiration driven N_2O flux from the soil through the plants to the atmosphere. The magnitude of any artifact will depend on the capacity of plants to transport N_2O and on soil conditions such as moisture and the dissolved N_2O concentration, whereas the significance of plant effects will depend on other components of the total N_2O flux derived from the soil surface. In the case of rice (*Oryza sativa* L.) plants, they need to be included within the chamber to fully account for N_2O that may be emitted via aerenchyma transport (Bertora, Peyron, Pelissetti, Grignani, & Sacco, 2018).

Smart and Bloom (2001) found that wheat leaves could emit N_2O during assimilation of nitrogen. The rate increased 10-fold when the nitrogen source was switched from ammonium to nitrate, and they found that N_2O production was associated with photoassimilation of nitrite in the chloroplast. This process is recognized in many plant species (Yu and Chen, 2009). Bruhn, Albert, Mikkelsen, and Ambus (2014) also reported that an abiotic ultraviolet-induced process may also lead to the release of N_2O from plant surfaces. Blocking sunlight with opaque chamber materials, therefore, may reduce this N_2O flux source. Nitrous oxide emissions from lichens and mosses have also been shown to be related to respiration via a robust N_2O/CO_2 ratio (Lenhart et al., 2015), and more recently this ratio has also been shown to hold for 32 plant species with no measurable effect of light on the N_2O emissions (Lenhart et al., 2019). Even sterilized plants in the absence of ultraviolet radiation emitted N_2O, leading the authors to conclude the N_2O emissions were derived from an abiotic process (Lenhart et al., 2019). However, the relative magnitude of plant-derived N_2O production, and plant-facilitated transport of soil-derived N_2O via stomata, remains to be further explored. Suffice to say, the magnitude of any plant derived N_2O fluxes will depend on plant species, the amount of biomass (leaf surface area) enclosed by the chamber, and the inorganic nitrogen forms in the soil and their amounts. The significance of any such effect will depend on the relative N_2O flux from the soil itself.

Few studies have examined the potential artifact(s)—or their potential magnitude—that may result from the use of opaque materials during chamber N_2O flux measurements. If plants are enclosed in transparent chambers, there is clearly a conflict between the need to insulate the chamber to limit air temperature changes, and a need to maintain solar radiation for plant function. Thus, researchers need to be aware of these issues when designing experiments specifically to look at plant effects on N_2O fluxes.

9 | ACTIVE HEADSPACE MIXING

Manual gas sampling and mixing of headspace air in non-flow-through, non-steady-state chambers can potentially affect soil surface gas exchange and lead to a bias in results (Christiansen et al., 2011; Liu & Si, 2009; Rochette & Eriksen-Hamel, 2008; Rochette & Hutchinson, 2005). Manual gas sampling of chambers is the most common method of sampling N_2O concentrations, with potential artifacts of manual sampling minimized by selecting appropriate sample volumes—for example, <1% of headspace volume.

However, modeling has shown that soil gas fluxes can be underestimated if the air inside the chamber is not constantly mixed during the enclosure period (Liu & Si, 2009). Fans have been used to mix headspace air in closed chamber headspaces, to overcome possible bias from vertical gas concentration gradients. Jørgensen et al. (2012) mixed the headspace of their chambers immediately prior to measurement, to eliminate vertical concentration gradients in chambers containing plants 60–110 cm high. For the same reasons, Bertora et al. (2018) advocate for fans when determining fluxes from chambers containing rice plants. However, few studies have specifically examined the effects of fan mixing in chambers, especially on N_2O fluxes. Rochette and Hutchinson (2005) showed that, for a 60-L square chamber without fans, the CO_2 flux was highly variable, but when a single fan was used, CO_2 flux determinations were generally higher than unmixed fluxes. However, the results were inconsistent over time, and no benefit was obtained from multiple fans (two or four).

Using sand beds, Christiansen et al. (2011) set up five reference methane (CH_4) fluxes (60–2,000 $\mu g\ m^{-2}\ h^{-1}$), and studied the effects of manual sampling with syringes and fans on headspace air mixing and subsequent flux determinations, using a 68-L unvented chamber. In nonmixed chambers (no fans), syringe sampling altered CH_4 concentrations inside the chamber, leading to a 36% underestimate of the measured reference fluxes. Comparisons of reference and measured CH_4 flux estimates improved when horizontally positioned fans (68 $m^3\ h^{-1}$) were used to mix headspace air. The fan speed (3,000 rpm, $8.0 \times 8.0 \times 2.5$ cm, 24 V, mixing rate of 68 $m^3\ h^{-1}$) did not induce mass flow of gas from the sand beds.

Christiansen et al. (2011) concluded that further research was required to fully understand the combined effects of chamber dimensions and mixing rates on estimated flux rates. As noted above, it is likely that headspace mixing is more important in tall chambers enclosing a larger amount of biomass (such as a mature cereal crop). Using the same experimental system as Christiansen et al. (2011), and CH_4 as the study gas, a static chamber comparison campaign examined 15 chamber designs: seven without a fan and eight with a fan (Pihlatie et al., 2013). The ratio of chamber fluxes to reference fluxes did not differ with or without the use of a fan, although the experimental setup precluded the determination of headspace mixing effects.

A study comparing three methodologies (eddy covariance, automated chambers, and static chambers) measured N_2O fluxes from an irrigated maize field over a wide range of emission intensities (Tallec et al., 2019). When N_2O fluxes were of moderate intensity, fluxes determined using automated chambers, which circulated air at ∼1 L min^{-1}, were higher than those determined using eddy covariance or static chambers, particularly when the crop was tall and developed and turbulence inside the vegetation was low. Tallec et al. (2019) assumed that the use of the fan, which was activated throughout the measurement period, increased turbulence in the chamber, thus altering the diffusive boundary layer conditions, resulting in an increased soil flux which, along with "pre-storage" of N_2O in the chamber due to the high walls (22.7 cm) during nonmeasurement periods, led to the enhanced N_2O fluxes (Tallec et al., 2019).

Similarly, Koskinen et al. (2014) found that chambers overestimated nighttime respiration fluxes (CO_2) from a forested peatland due to the buildup of CO_2. This occurred because of the disturbance of the soil–moss CO_2 gradient and consequent initial pulse of CO_2 to the chamber headspace: besides evaluating data fitting methods, it was concluded that the fan be kept on at a minimum speed to avoid the pulse effect and ensure mixing.

In theory, the perfect mixing system should align headspace mixing intensity with pre-deployment conditions (Rochette & Hutchinson, 2005). This is not a simple task to achieve, but it suggests that placement of nonmixed chambers in an exposed windy environment—and of strongly mixed chambers in calm locations (i.e., under a plant canopy)—would result in the greatest flux measurement biases.

Pihlatie et al. (2013) assumed that the earlier results obtained by Christiansen et al. (2011) would also apply for a larger group of chambers: the use of a fan improves the flux estimate and decreases the uncertainty in the flux. This sentiment is echoed by Hoffmann et al. (2018), who noted that although a well-sealed chamber determines the flux measurement accuracy, the mixing of the headspace with a fan improves measurement precision. However, Hoffmann et al. (2018) also warn that if the chamber seal is not airtight at the chamber–base interface, the diffusive chamber leakage will be reinforced by use of a fan that will then induce mass flow, leading to bias flux determination. Pihlatie et al. (2013) noted that further studies dedicated to examining CH_4 and N_2O fluxes, as a result of headspace mixing, and the speed of headspace mixing, are needed. Since this publication, some studies have provided additional information. Using recirculating flow through chambers to study manure- and soil-derived emissions, Parker et al. (2017) and Christiansen et al. (2011) found that the use of an internal fan improved the precision of the measurement and reduced the CV by an order of magnitude, but increased the N_2O flux relative to no fan being used. However, the argument can also be made that airflow within the chamber is more representative of external conditions where wind events do occur. Korkiakoski et al. (2017) used automated chambers (97 L) equipped with fans (24 V, size = 8 cm × 8 cm, operated constantly at minimum speed) to measure CH_4 uptake in a peatland forest floor, and where a wind-induced diel cycle in CH_4 uptake was observed, implying that over- or underestimation of the actual CH_4 uptake flux during lower or higher fan speeds would occur. Again, this led to the conclusion that gas fluxes would be improved if fan speed varied with ambient wind speed in order to better mimic variations in atmospheric mixing (Korkiakoski et al., 2017). In general, more information is still required to better ascertain how chamber geometry and fan speed affect the diffusive boundary layer and resulting N_2O flux.

The use of fans in chambers must be accompanied by laboratory bench testing (e.g., Hoffmann et al., 2018) prior to field use. Fans should be mounted so that the airflow is across the soil surface (not directly into or out of the soil). Pavelka et al. (2018) reported that the average speed of air movement inside the chamber should be <0.5 m s^{-1}, measured at four points across the chamber and at half the height of the chamber. However, it is unclear how this value is derived. Similarly, when reporting results, the fan size, make, model, applied voltage, and ensuing wind speed or mixing rate should all be reported. At present, the literature is insufficient to make specific recommendations on the relative size requirements and speed of fans.

10 | SUMMARY

As noted by Pavelka et al. (2018), standard chamber design is about refining and customizing the design to the given ecosystem under study so that the design and measurement protocol minimizes potential artifacts. Factors to consider when optimizing such protocols are summarized in Table 1. Initially, researchers must consider the objectives of the experimental program in which the chambers will be used, the nuances of the ecosystem under study, and the soil characteristics at the intended site(s). This—along with the principles outlined above, and further research to fine-tune them—will produce an optimal chamber design. Before deployment, the chosen chamber design should be "bench tested" on an impermeable surface to ensure

TABLE 1 Summary of considerations when designing non-steady-state chambers

Design feature	Design objective	Minimum requirements	Site-specific issues	Evolving issues
Materials	To prevent gas exchange through chamber.	Inert to N_2O, such as stainless steel, aluminum, polyvinyl chloride (PVC), acrylic.	Robust frames required to withstand grazing.	
Area	Minimize error due to poor sealing and maximize area sampled.	A chamber area/perimeter ratio of ≥10 cm is recommended (equates to a cylindrical chamber of ≥40 cm diameter).	Adaption needed if rocks or roots are present, or if required by research objectives.	
Height	Maximize flux detection and minimize perturbation of environmental variables.	Chamber height (cm) to deployment time (h) ratio should be ≥40 cm h^{-1}.	Chamber height should accommodate crop height.	
Base depth	Prevent below ground lateral gas transport, shading and ponding of water.	Ratio of insertion depth: to deployment time of ≥12 cm h^{-1}. Height above soil surface should be as close to the soil surface as practical (<5 cm).		
Gastight seal	Prevent gas leaking between chamber and base.	A water trough or rubber/closed cell foam gasket. Gaskets should have low internal cross-sectional area and be compressible; appropriate fasteners are required with rubber gaskets.		
Sampling port	For extracting sample.	Inert rubber septa or syringe taps		
Vent while placing chamber on base	To prevent pressure disturbance while placing the chamber on the base.	Opening a vent or sampling port *while placing the chamber* is essential.		
Vent during deployment	To prevent pressure gradients between the interior and exterior of the chambers during flux measurement and gas sampling.	Tube-type vents should be located close to the soil surface, or be designed to minimize wind effects. Appropriate vent dimensions (diameter and length) are dependent on expected wind speeds during deployment and should be adjusted accordingly (see references in text). Chambers and their vents should be bench tested to ensure no Venturi effect occurs. Designs exist to overcome Venturi effects.		
Insulation	Prevent temperature gradients between the interior and exterior of the chambers.	Use reflective foil, foam, or polystyrene. Test effectiveness by comparing surface soil temperatures inside and outside the chambers		

(Continues)

TABLE 1 (Continued)

Design feature	Design objective	Minimum requirements	Site-specific issues	Evolving issues
Headspace mixing	Well-mixed headspace to ensure that representative sample is taken.	Active headspace mixing (e.g., fans) should not affect the diffusive flux.	Crop type and chamber height.	Effects of mixing should be tested and reported on. There has been relatively little work performed on evaluating specific requirements for given chamber geometries and fan size–wind speed combinations.

that materials are inert, that there are no leaks or Venturi effects at anticipated deployment wind speeds, and that possible temperature perturbations have been eliminated. Plants inside chambers create unique challenges: if the aim is to maintain plant function during the enclosure period, chamber design needs to be carefully considered. Finally, significant vertical gradients may develop within the chamber in certain deployment applications, and further studies are needed to assess the best way of alleviating these prior to, or during, sampling.

ACKNOWLEDGMENTS

This work was funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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How to cite this article: Clough TJ, Rochette P, Thomas SM, Pihlatie M, Christiansen JR, Thorman RE. Global research alliance N_2O chamber methodology guidelines: Design considerations. *J. Environ. Qual.* 2020;49:1081–1091. https://doi.org/10.1002/jeq2.20117